Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage:www.elsevier.com/locate/apjtb

Document heading

Hemolymph proteins in marine crustaceans

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ARTICLE INFO

Article history:
Received 208 September 2011
Received in revised form 2 November 2011
Accepted 10 December 2011
Available online 28 June 2012

Keywords:
Antimicrobial peptides
Crabs
Antibiotics
Protein characterisation
Immune response

ABSTRACT

This study is done with the aim to bring together the various antimicrobial peptides that are present in the crustacean hemolymph and their sources along with its characteristics. Invertebrates lack immune systems that involve antigen-antibody reactions and do not have an immune memory, therefore most invertebrate species show no evidence of acquired immunity. Crustaceans possess an open circulatory system, where nutrients, oxygen, hormones, and cells are distributed in the hemolymph. They lack adaptive immune system and rely exclusively on their innate immune mechanisms that include both cellular and humoral responses. Antimicrobial peptides and proteins form an important means of host defense in eukaryotes. In addition to their role as endogenous antibiotics, antimicrobial peptides have functions in inflammation, wound repair and regulation of the adaptive immune system. Over the past several years, many antimicrobial peptides have been found and characterized in crabs.

1. Introduction

Crustaceans form a very large group of arthropods, usually treated as a subphylum, which includes familiar animals such as crabs, lobsters, crayfish, shrimp, krill and barnacles. More than 10 million tons of crustaceans are produced by fishery or farming for human consumption, the majority of it being shrimps and prawns. Hemolymph, is a fluid in the circulatory system of some arthropods. Hemolymph fills all of the interior (the hemocoel) of the animal's body and surrounds all cells. It contains hemocyanin, a copper-based protein which turns blue in color when oxygenated, instead of the iron-based hemoglobin in red blood cells found in vertebrates, thus giving hemolymph a blue-green color rather than the red color of vertebrate blood.

Naturally occuring agglutinating materials from the hemolymph of numerous invertebrates have often been reported. It seems quite clear that these proteins are not immunoglobulins, although in their biological activity, they may resemble vertebrate antibodies. Although the details of some of these chemical interactions are increasingly available, the basic biological usefulness of the materials stays unknown. Studies on the immunity of invertebrates have been focussed on identifying defence mechanisms and biochemical pathways activated during an infection, and on identifying cell-free hemolymph and cellular factors involved in the destruction of pathogens, regulation, and damage repair. Crustaceans possess an open circulatory system, where nutrients, oxygen, hormones, and cells are distributed in the hemolymph[1]. Crustaceans lack adaptive immune system and they rely exclusively on their innate immune mechanisms that include both cellular and humoral responses[2].

Research in immunology of commercially important marine invertebrates is currently related to infectious pathology but is progressively drawing nearer to genetics, on the one hand to characterize the genes of defence response effecters, and on the other hand to select pathogen-resistant strains, either by quantitative genetics or by genetic transformation[3].

2. Antimicrobial peptides and proteins

Antimicrobial peptides are important members of the host defense system. They have a broad ability to kill microbes. Large antimicrobial proteins (>100 a.a.), are often lytic, nutrient-binding proteins or specifically target microbial macromolecules. Small antimicrobial peptides act by disrupting the structure or function of microbial cell

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Foundation Project: This work was financially supported by University Grants Commission, Government of India.

membranes. A multitude of antimicrobial peptides have been found in the epithelial layers, phagocytes and body fluids of multicellular animals including man. In addition to their role as endogenous antibiotics, antimicrobial peptides have functions in inflammation, wound repair and regulation of the adaptive immune system. The various antimicrobial peptides and their sources are tabulated in Table 1.

Antibacterial peptides can also be induced in epidermal cells in response to wounding or infection in the cuticles[4]. The whole process of synthesizing antibacterial proteins may take few minutes or hours after the challenge and these are secreted into hemolymph when invertebrates are in acute phase. Most of these proteins are small cationic molecules exhibiting a broad spectrum of activity against gram-positive and/or gram-negative bacteria. There are some antibacterial proteins that are not inducible such as lysozyme[5] and andropin[6]. Over the past several years, many antimicrobial peptides have been found and characterized in crab species. The first antimicrobial peptide characterized was a proline peptide of 6.5 kDa from the hemocytes of the shore crab *Carcinus maenas*[7]. The antimicrobial peptide callinectin is a cationic antimicrobial peptide of 3.7 kDa isolated from the blue crab *Callinectes sapidus*[8]. Recently, scygonadin, an anionic antimicrobial peptide has been isolated from seminal plasma of the mud crab *Scylla serrat*[9]. More recently, antilipopolysaccharide factors (ALFs), originally identified from the amoebocyte of the horseshoe crab *Limulus polyphemus*[10,11] have been identified from the hemocytes of several species of decapods including *Fenneropenaeus chinensis*[1], *Marsupenaeus japonicas*[12], *Penaeus monodon*[13], *Litopenaeus setiferus*[14], *Pacifastacus leniusculus*[15], *Carcinus maenas*, *Callinectes sapidus*[16], *Eriocheir sinensis*[17] and *Scylla paramamosain*[18].

2.1. Lectin

Lectins are naturally occurring proteins or glycoproteins which bind selectively and noncovalently to carbohydrate residues. The main characteristic of this class of protein is their ability to interact specifically with carbohydrates and combine with glyco-components of the cell surface[19]. Lectins from the hemolymph of invertebrates, including crustaceans, have been regarded as potential molecules involved in immune recognition and microorganism phagocytosis through opsonisation. These proteins, considered as functional precursors of antibodies, constitute a group of proteins generically denominated lectins. Lectins in cell-free hemolymph have been identified in almost all crustaceans. Moreover, lectins with structural characteristics and identical specificity to the cell-free hemolymph lectins, have been identified in the hemocyte membrane and in cytoplasmic granules[20]. In crustaceans, sialic acid-specific lectin of Indian horseshoe crab, *Carcinoscorpius rotunda cauda*, could agglutinate many types of bacteria and a lectin identified from the hemolymph of the blue crab *Callinectes sapidus* has also been found to possess agglutinating activity against several serotypes of *Vibrio* spp.[21-23]. The relevance of these lectins in the host defense system relies on the observation that sialic acid, an important constituent of many glycoconjugates, is present on different cell surfaces[24,25]. The selective binding of hemolymph lectin to some of the shrimp pathogenic bacteria suggests that the lectin plays a role in the defense against these pathogenic bacteria.

2.2. Callinectin

The blue crab, *Callinectes sapidus*, is a decapod crustacean of the brachyuran family Portunidae. Callinectin has broad-spectrum of antibacterial activity in its hemolymph that constitutes part of its nonspecific defences. Callinectin from blue crab hemocytes was purified using its basic (cationic) nature. Purified callinectin was active against *Escherichia coli* D31. Blue crab hemolymph has potent, broad-spectrum, antibacterial activity against many gram-negative organisms, including *Vibrios* and aeromonads. Callinectin's predominance as an antibacterial factor in blue crab hemocytes suggests that it plays a major role in blue crab immunity. The antibacterial activity of blue crab hemolymph is severely depressed in polluted waters[26]. Thus, callinectin

Table 1
Various antimicrobial peptides and its sources.

S.No	Protein	Molecular weight (kDa)	Species
1	Callinectin	3.7	*Callinectes sapidus*
2	Scygonadin	43.0	*Scylla serrata*
3	ALFs	-	*Limulus polyphemus*
4	Lectin	-	*Carcinoscorpus rotunda*, *Callinectes sapidus*
5	Apoprotein	-	Shrimp
6	Cryptocyanin	-	*Cancer magister*
7	Phenol oxidase	300.0	*Panulirus argus*
8	Crustins	7.0-14.0	*Hyas araneus*
9	Scyllin	4 000.0	*Scylla serrata*
10	Artemocyanin	-	*Streptocephalus*, *Leptestheria*
11	Penaeidin	5.5-6.6	*Litopenaeus vannamei*
12	Clottable protein (CP)	400.0	*Penaeus vannamei*
13	Pernin	60.0	Mytilidae
14	Glycoproteins	-	*Tigriopus japonicus* and *Coullana* spp.